CCD camera. Excitation light from a full-field White Lite® light 300 W xenon arc lamp was bandpass filtered using a 525 nm excitation filter (Omega Optical Inc, VT) and focused uniformly on the sample using a set of two optic fiber cables (mellesgriot) held at an angle of 45 degrees. The fluorescent spots were focused onto the CCD using a camera lens (Infinimite® alpha, Edmund Optics) and filtered using a 600 nm longpass filter. Custom algorithms, built within the Lumenera camera software corrected for CCD dark noise. Images saved in tiff format were analyzed using the Scanarray Express™ software (Perkin Elmer, Wellesley, Mass.).

Results

A method was developed that requires the addition of only one fluid sample and can therefore measure multiple biomarkers simultaneously in one simple process. This technology showed a potential to facilitate common use of antibody microarray in medical and scientific field for high-throughput detection of a wide variety of analytes.

Lateral Flow Assays

To achieve rapid, multiplexed detection, an LFA (as shown in FIG. **19**) was developed which combines the multiplexed, quantitative advantages of the protein microarrays and the assay speed and simplicity of traditional LFA. Multiple capture molecules were spotted onto the nitrocellulose membrane, and the reporter mixture was applied to the conjugate pad at one end of the membrane. This complex was drawn through the membrane by capillary action, where the markers were captured by their respective ligands. This simple, yet rapid method required the addition of only one fluid sample without the need for washes. Fluorescent QD nanocrystals were employed as the reporter since they have an added advantage of being multiplexed, yet quantitative. The use of spectrally different QDs as well as spatial separation of these two capture molecules enables reliable multiplexed detection in this lateral flow format.

To demonstrate the proof of concept of multiplexed lateral flow assays, two test analytes (Biotin-BSA and mouse IgG) were detected on a single LFA. As shown in FIG. **20**, when a mixture containing streptavidin conjugated QD 585 and Goat anti-Mouse conjugated QD 605 (both at 10 nM) is added to the conjugate pad (left side of the FIG.), it flows through the pores of the nitrocellulose membrane, to the capture zones where the streptavidin conjugated QD 585 binds to the biotinylated BSA and Goat anti-Mouse conjugated QD 605 binds to the mouse IgG respectively. Since the capture molecules are fixed on the membrane, the reporters continuously accumulate on the capture zone. This generates a signal proportional to the amount of immobilized capture molecules. FIG. **20**(A) demonstrates a strip with biotin-BSA spotted as two consecutive 1 mm (100 nl) lines along the length of the nitrocellulose membrane at a concentration of 500 μg/ml and at 125 μg/ml in FIG. **20**(B). In order to measure multiple analytes along the strip, it is important that the flow not be obstructed by the capture molecules. It is observed that with a capture concentration of 500 μg/ml very little signal is obtained from the second line. However, when the concentration of the capture molecule is reduced to 125 μg/ml, some signal is observed on the second line. When the concentration is kept high (500 μg/ml), but the dispensing volume is reduced to 20 nl, five columns of capture molecule can be observed (FIG. **20**(C)). However, in this case, a reduction of signal intensity is observed in the direction of sample flow. When this capture volume is reduced to 10 nl (FIG. **20**(D)), this gradation disappears and the spots have uniform fluorescence signal along the length of the membrane. The result suggested the possibility that multiple target proteins could be detected by adjusting the amount of capture antibody on the strip. To demonstrate this multiplexing capability of the LFA, one column (3 spots) containing biotin-BSA was immobilized and a second column (3 spots) containing mouse IgG (FIG. **20**(E)). Two spectrally distinct sets of QDs were used for this assay and the mixture containing the two different detectors is accurately resolved on the LFA demonstrating its multiplexing capability.

The microarray LFA was then employed for the detection of the panel of breast cancer biomarkers including Her-2, CA 15-3 and Osteopontin. Monoclonal capture antibodies to the four antigens were immobilized on the nitrocellulose membrane as 10 nl spots. This spotting was done in six schemes such that the sequence in which the sample encountered the capture molecules was different in each assay. A mixture of antigens and biotinylated detector antibody labeled with streptavidin QD 605 as deposited on the conjugate pad. The concentrations of reagents used in this assay are Her-2 5 μg/ml, CA 15-3 500 U/ml and Osteopontin 3 μg/ml, biotinylated detector antibodies 30 μg/ml and strepavidin QD 605 10 nM. The solution was allowed to wick through the membrane and the spot fluorescence was observed on the UV transilluminator. Images were captured using a digital camera and displayed in FIG. **21**, which shows that it is possible to observe signals from three antigens in this multiplex format. The signal to noise ratio was best for the case (C) and (E) where Her-2 is spotted at the far right hand side of the membrane. Although high concentrations of antigens were used, signal was barely observed above the high background in the membrane.

Accumulation of fluorescent quantum dot in the pores of the nitrocellulose membrane, as well as the high concentration of reagents in this assay cause a high background noise in the LFA. The LFAs had much lower sensitivity than that of the microarrays. The concentrations of capture and biotinylated detector antibodies were 8 fold and 6 fold higher than those used in the protein microarrays respectively, making the assay very expensive. Although the Her-2, CA 15-3 and OPN were detected in a multiplexed format, MMP-2 assay did not produce any signals on the LFA. The detection limits of the LFA for the antigens were approximately 10 fold higher than protein microarrays.

The reagents for the multiplexed assay were optimized specifically for the protein microarray platform as discussed above. By optimizing a brand new set of reagents for the LFA, it could improve the sensitivity of the assay as well as make MMP-2 work with the panel of biomarkers. The use of a membrane substrate with high affinity to proteins made LFAs prone to very high background noise. This is partially due to the fact that LFAs do not involve wash steps, and that the flow of analyte solution is through the membrane and not simply above the surface. This offers a three dimensional matrix to which the analyte, detector antibody and reporter complexes can bind non-specifically. Unlike the traditional Western Blots, ELISAs or Microarrays, the blocking agents employed to minimize the background levels bind to the membrane matrix and offer resistance to the flow of analyte through membrane. Additionally, such additives can displace capture reagents from the membrane, thereby, reducing assay sensitivity.

Channel Flow Assays

To design a new platform for rapid immunoassays, the antibody arrays were printed on a glass slide and instead of the static incubation chambers, unique flow channels were designed that cover individual arrays, and allow for passive flow of analyte mixture over the immobilized array. Channels are made by using adhesive silicone supports and a glass cover slip. This helps draw fluids onto the immobilized anti-